United States Department of Agriculture
Animal and Plant Health Inspection Service
Center for Veterinary Biologics
P. O. Box 844
Ames, IA 50010

1. Reagent Name: *Escherichia coli* Anti-K99 Pilus Monoclonal Antibody (K99 MAb)

2. Strain or Source: Hybridoma 2BD4E4

3. Lot Number: IRP 557 (07)

4. Fill Date: May 18, 2007

5. Expiration Date: Not Applicable.

Precautions: There are no known hazards associated with this reagent.

6. Intended Use: For use in potency testing of *E. coli* biologicals containing the K99 antigen, according to **Supplemental Assay Method (SAM) 620**.

7. Instructions for Use: Dilute the K99 MAb 1:5,000 (or as optimized in specific titration studies) in cold carbonate-bicarbonate coating buffer (0.05M, pH 9.6) and use immediately according to **SAM 620**. Dilute to final dilution by adding 10 µL undiluted MAb to 9.99 mL buffer (1:1000), then prepare final dilution by adding 2 mL (1:1000) to 8 mL buffer to prepare the 1:5000.

8. Test of Reagent: The K99 MAb was shown to be specific for K99 pilus antigen, and it demonstrated minimal nonspecific binding (background) in assays performed according to **SAM 620**.

9. Container Size, Type, Weight, or Volume: 150 µL aliquots in 0.5-mL microfuge vials (sufficient for 12-15 plates).

10. Storage Conditions: -20° to -80°C for long-term storage. A vial may be held at 2°- 7°C for several months.

11. CVB Technical Contact: Bacteriology Section, Center for Veterinary Biologics, (515) 337-6140 or FAX (515) 337-7673.

12. Origin and Passage History: The hybridoma cell line secreting anti-K99 antibody was obtained from ATCC.

13. Method of Preparation: The hybridoma cell line was propagated in a bioreactor to produce supernatant fluid containing K99 MAb. The 500 mL harvest was originally frozen at -70°C or lower on December 23, 2002. The antibody was thawed in May of 2007; 75 mL was removed and aliquoted into 150 µL/vial. This lot was designated 2BD-001.

14. Other:

Restrictions: This reagent is to be used only in conjunction with biological potency testing according to SAM 620.

Reagent orders and feedback should be sent *including phone number* to the following email address: CVB@aphis.usda.gov

Reagent orders forms (APHIS 2018) are available from:
http://www.aphis.usda.gov/animalhealth/cvb_forms

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